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Effects of Sulfur Dioxide on Catalase Activity in Plants

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植物體의 Catalase 活性에 미치는 아황산가스의 影響

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Abstract

The experiments were conducted to examine the effects of sulfur dioxide on catalase activity in germinating seeds under dark conditions and in green plants under light conditions. Three kinds of plants, bean (*Glycine max* M.), cabbage (*Brassica oleracea* L. Chungbang No.1) and rice (*Oryza sativa* L. Suwon No.213—1) were used in this study. Both the germinating seeds and the leaves were treated with various concentrations of sulfur dioxide for both one green plants with two and twelve hours.

In the results of the measurement of catalase activity it was found that plant injuries by sulfur dioxide for the period of photosynthesis were higher than those for the period of respiration. In the control, apparent catalase activity under the light condition of photosynthesis showed a considerable decrease in comparison with catalase activity under the dark condition of respiration. This tendency also appeared markedly in the gas treatment.

In the gas treatment for twelve hours, the decrease of catalase activity was higher than that of one hour in both photosynthesis and respiration. It was thought that sulfur dioxide was an inhibitor of catalase activity in higher concentration of the gas.

Introduction

In a previous paper (Sung, 1973) it was reported that ammonia reduced the sulfur dioxide injuries in plants. Numerous reports have been written on sulfur dioxide; absorption, distribution, amino acid synthesis in a low concentration(Fumia & Hirokazu, 1972), and photosynthesis and respiration in plants (Thomas and Hill, 1937).

Recently, Taniyama, et al. (1972) have reported a considerable decrease of photosy-nthesis and a decrease of dark respiration during the treatment of sulfur dioxide. On the other hand, Amberger and Wünsch(1963) have reported that cyanamide was an inhibitor of catalase. In general terms, catalase destroys hydrogen peroxide which cells

produce as the result of metabolism, and, protects all plants and animals from the toxicity of hydrogen peroxide accumulated in cells(Chance, 1951; Martin Frobischer, 1968).

Though catalase inhibitors have been known as NH_3, H_2S, HCN, NH_2OH (Keilin & Hartree, 1945), sulfur dioxide has not been known as catalase inhibitor. Plant injuries from the more active combination of hydrogen peroxide produced in cells and sulfur dioxide entering from the stomata seem to be higher than those from the active combination of water and sulfur dioxide. Therefore, this study was conducted to investigate the effects of sulfur dioxide on catalase activity in germinating seeds and green plants.

Materials and Methods

Effects of sulfur dioxide on catalase activity in the germinating seeds.

Three kinds of plants, bean (*Glycine max*(L) Merril Syn.), cabbage (*Brassica oleracea* L. Chungbang No. 1) and rice (*Oryza sativa* L. Suwon No. 213—1.) seeds were used as the materials for this study, between June and September, 1973.

In one experiment with germinating seeds, the plant seeds were germinated in a 400m*l* beaker spread with one layer of filter paper, dropped in 4m*l* of Hoagland solution, covered the beaker with polyethylene sheet and transferred the beaker to an incubator at 25°C for five days. The germinated seeds in the beaker were fumigated with 0, 1, 5, 10 and 50ppm SO_2, the same methods as a previous paper (Sung, 1973), in a dark condition of respiration for both one and twelve hours.

The germinating seeds treated with the gas were collected, weighed 1g each according to the gas concentration and ground in a mortar into which was dropped 5m*l* of 0.07M phosphate buffer solution. The ground materials were filtered and stored in a refrigerator at 0°C in order to prevent the catalase from being destroyed. This stored solution was used as a enzyme solution for the measurement of catalase activity.

The catalase activity was measured by the modified methods of Euler and Josephson(1923) as following Fig. 1.

Materials, 1g
↓
Phosphate buffer solution, pH 6.8
↓
Ground in mortar, 3 minutes
↓
Filteration at 0°C
↓
Stored in refrigerator at 0°C
↓
Enzyme solution ⟶ 1ml of enzyme solution
↓
The modified Warburg manometer
↓
Addition of 4ml 3% H_2O_2
↓
Water bath at 30°C, 30 minutes
↓
Oxygen evolution

Figure. 1. Procedure of the extraction of enzyme solution and the measurements of catalase activity as the volume of oxygen evolution.

Effects of sulfur dioxide on catalase activity in the green plants.

The above mentioned germinating seeds were cultured in the water culture bottle with Hoagland solution for ten days. The green seedlings were washed with distilled water, and each weighed one gram. They were placed in a 400 m*l* beaker which contained a layer of filter paper. The culture solution of 4 m*l* was dropped in the beaker and was covered with two layers of polyethylene sheet. The treatment of the gas were conducted with a micro-injection.

In one earlier experiment, the beaker was transferred to the incubator at 25°C, 95% humidity under the dark condition of respiration for both one and twelve hours. In the other later experment the beaker was transferred to the incubator table at 25°C, 95% humidity under the light condition of photosynthesis. The light condition was conducted with the illumination of 30 watt light at a distance of one meter for both one and twelve hours.

After the treatment of gas to the plants in the beaker, under the dark or light conditions for both one and twelve hours, the green plants with two leaves were removed from the beakers and each weighed one gram according to each of the

gas concentrations. The weighed materials were ground rapidly in a mortar. Catalase activivity were measured with the same methods as Fig. 1.

Results and Discussion

Effects of sulfur dioxide on catalase activity in the germinating seeds.

Fig. 2—6 showed the results of the effects of sulfur dioxide on catalase activity in the germinating seeds. In the study of catalase activity in the germinating seeds by the treatment with sulfur dioxide for one hour, as the concentrations of sulfur dioxide were increased, catalase activity decreased. As compared with the control, catalase activity of bean was highest, but that of rice was lowest. This tendency of catalase activity was different according to the germinating periods and the kinds of seeds.

The germinating seeds conducting respiration were effected by sulfur dioxide. Therefore, catalase activity of the germinating seeds was inhibited by a concentration of more than 1 ppm of sulfur dioxide. It seemed that sulfur dioxide in the germinating seeds acted as an inhibitor of catalase. Taniyama, et. al. (1972) have repo-rted that sulfur dioxide decreased dark respiration in rice plants. Agner and Theorell(1946) have reported that catalase activity was affected markedly by hydrogen ion. In this study it was though that at first, the accmulation of hydrogen ion by sulfur dioxide and the acidity of cytoplasm in germinating seeds might be able to make catalase activity decrease (Sung, 1973).

Fig. 3 showed catalase activity in the germinating seeds by the treatment with sulfur dioxide for twelve hours. In Fig. 4—6, the decrease of catalase activity effected by sulfur dioxide for twelve hours always showed a higher value than that of one hour. In the treatment of sulfur dioxide for twelve hours, catalase activity was stopped with 10—50ppm of sulfur dioxide. As sulfur dioxide concentrations were increased, apparent catalase activity decreased proportionally. It was thought that the gas was dissolved, acidified rapidly in cytoplasm of the germinating seeds by passing through in time and inhibited catalase

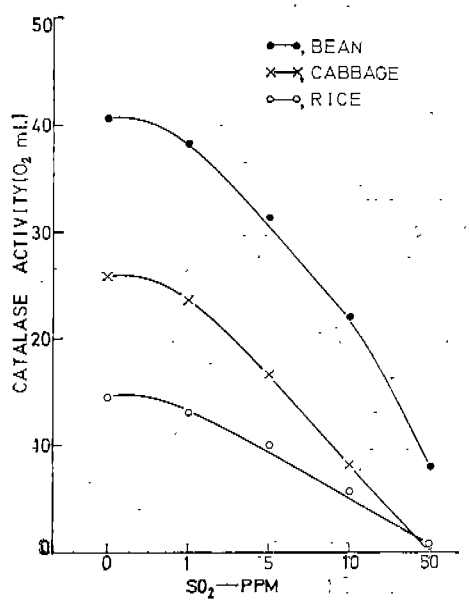

Fig. 2. Catalse activity according to the kinds of germinating seeds treated with SO_2 concentrations for one hour.

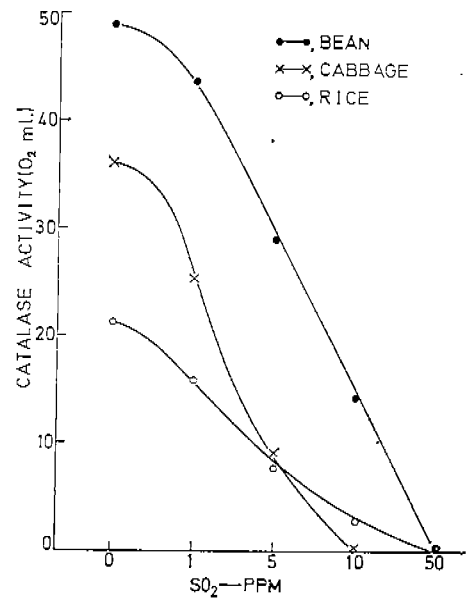

Fig. 3. Catalase activity according to the kinds of germinating seeds treated with SO_2 concentrations for twelve hours.

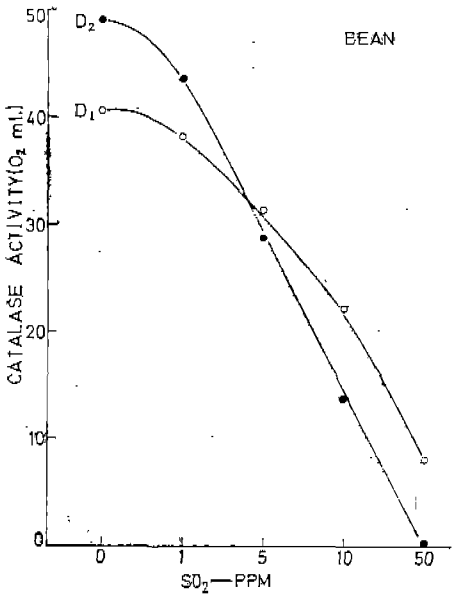

Fig. 4. Catalase activity in dark respiration of the germinating bean seeds treated with SO_2 concentrations for one (D_1) and twelve (D_2) hours.

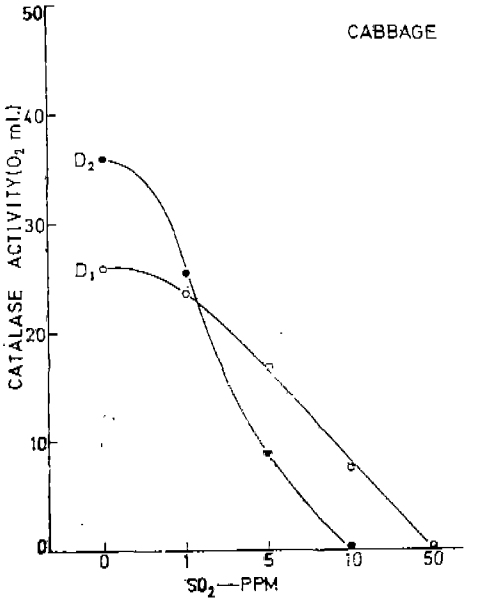

Fig. 5. Catalase activity in dark respiration of the germinating cabbage seeds treated with SO_2 concentrations for one (D_1) and twelve (D_2) hours.

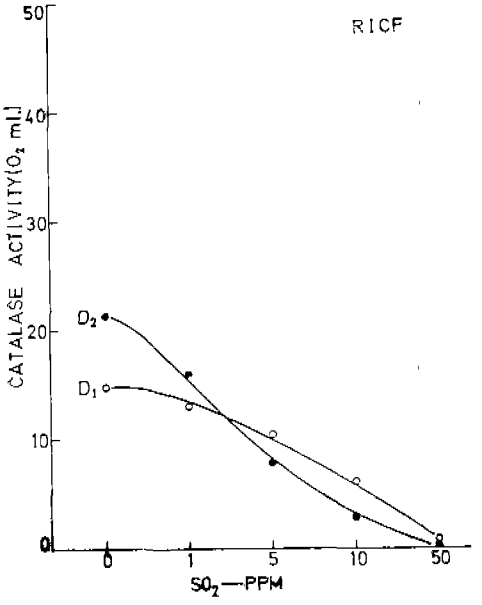

Fig. 6. Catalase activity in dark respiration of the germinating rice seeds treated with SO_2 concentrations for one (D_1) and twelve (D_2) hours.

activity. Moreovver, when the germinating seeds conducted aerobic respiration, the gas seemed to operate as an inhibitor of catalase.

Effects of sulfur dioxide on catalase activity in the green plants.

In Fig. 7—9, catalase activity in the control of photosynthesis were decreased more than in the dark respiration. As sulfur dioxide concentrations were increased, apparent catalase activity for the period of photosynthesis and respiration decreased proportionally. In the green plants treated with sulfur dioxide for twelve hours, the decrease of catalase activity always higher values than that of one hour.

Especially, the decrease of catalase activity effected by the gas under the light condition of photosynthesis was more apparent than that under the dark condition of respiration. This is because the stomata of the green plants are opened for photosynthesis under the light condition and are closed for respiration under the dark condition. In the treatment of the gas for twelve hours it

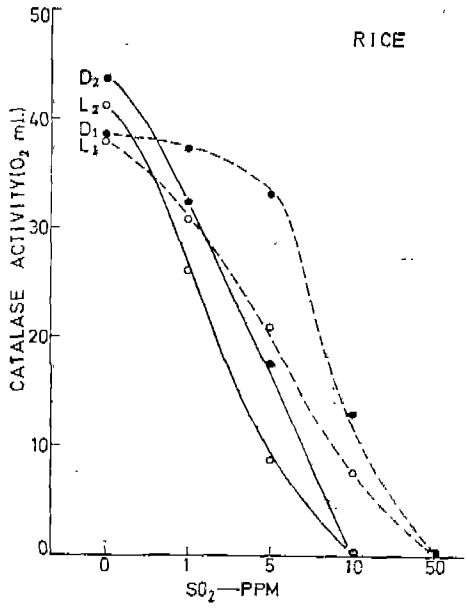

Fig. 7. Effects of SO_2 on catalase activity in photosynthesis and dark respiration of bean leaves treated with SO_2 concentrations according to light (L) and dark (D) conditions for one (D_1, L_1) and twelve(D_2, L_2) hours.

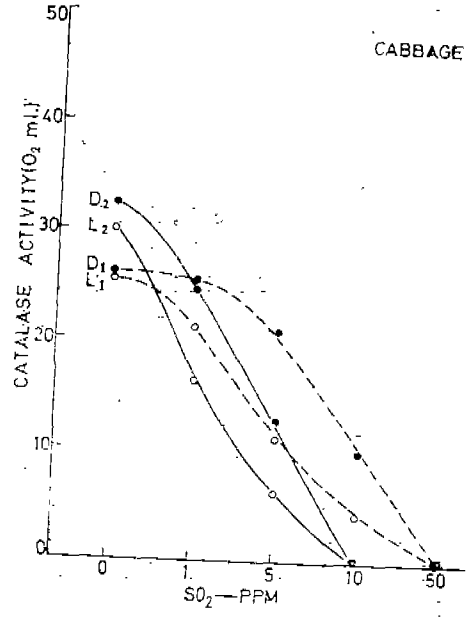

Fig. 8. Effects of SO_2 on catalase activity in photosynthesis and dark respiration of cabbage leaves treated with SO_2 concentrations according to light (L) and dark (D) conditions for one (D_1, L_1) and twelve (D_2, L_2) hours.

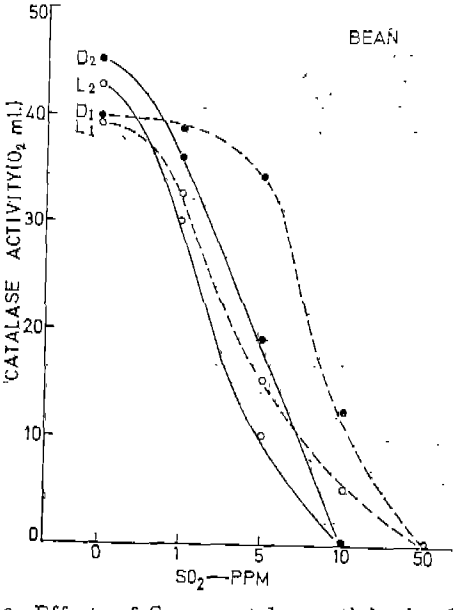

Fig. 9. Effects of So_2 on catalase activity in photosynthesis and dark respiration of rice leaves treated with SO_2 concentrations according to light (L) and dark (D) conditions for one (D_1, L_1) and twelve (D_2, L_2) hours.

stopped at 10ppm of sulfur dioxide. This tendency was always same in the bean, cabbage and rice whether concerning photosynthesis or resperation.

Amberger and Wünsch(1963) have written that cyanamide was rapidly taken up by plants, resulting in a marked inhibition of catalase within a few hours, first in the roots and then in the leaves. And Fumia & Hirokazu(1972) found that distribution of $^{35}SO_2$ entered into plant leaves from the atmosphere was highest in leaves, and then stem, and was lowest in the roots. In this study, sulfur dioxide was rapidly taken up by the germinating seeds and resulted in a marked inhibition of catalase within one hour. Although catalase inhibitors were known as KCN, NaF, NO, NaN_3, NH_3, NH_2 OH and many anions (Agner & Theorell, 1946), little is known about the catalase inhibitor of sulfur dioxide during photosynthesis and respiration in higher plants.

Hydrogen peroxide is produced by many cells, especially aerobics, rarely anaerobic cells (Martin

Frobischer, 1968). Catalyzing the breakdown of hydrogen peroxide by catalase, the catalase may serve only to protect the organisms against hydrogen peroxide or may have a broader function in the oxidation of organic molecules (Keilen & Hartree, 1945; Chance, 1951). If catalase activity was inhibited by sulfur dioxide in cells, the cells would be killed by the accumulation of hydrogen peroxide with strong activity. Sulfur dioxide easily could combine with hydrogen peroxide than water in cells.

$$H_2O_2+SO_2 \longrightarrow H_2SO_4$$

Therefore, hydrogen peroxide and sulfur dioxide could make sulfuric acid in many cells with catalase. Then the living organisms would become poisoned by the sulfuric acids and would be killed. If sulfur dioxide were combined with hydrogen peroxide in cells, the toxicity of catalase would be higher than the combination of sulfur dioxide and water. Because the combination of sulfur dioxide and hydrogen peroxide in cells is rapid in comparison with that of sulfur dioxide and water.

$$H_2O+SO_2 \longrightarrow H_2SO_3$$
$$H_2SO_3+O_2 \longrightarrow H_2SO_4$$

Both sulfur dioxide and hydrogen peroxide are bleaching chemicals. They easily bleach the tissues of living organism. Therefore, it was thought that the decrease of catalase activity by sulfur dioxide might be able to increase the accumulation of hydrogen peroxide in cells; and, the toxicity of sulfur dioxide could be increased rapidly.

摘 要

SO_2로 因한 植物體의 被害는 SO_2가 植物體內에 들어와 水分과 作用하여 黃酸을 形成하기 때문이라고 認識되어져 있다. 그러나 SO_2가 植物體의 物質代謝 結果로 生成되는 過酸化水素와 反應하여 黃酸을 形成하는 作用이 더욱 活性的이므로 그로 因한 被害는 加速化된다고 生覺되었다.

그러므로 배추(*Brassica oleracea* L. 청방 1호), 벼(*Oryza sativa* L. 수원 213—1) 및 콩(*Glycine max* Merr.)을 材料로 發芽種子와 生長한 綠色植物에 SO_2를 몇가지 濃度別로 處理하여, SO_2가 植物體에 미치는 被害 機作의 一部로서 catalase 活性에 미치는 影響을 調査한 結果는 다음과 같다.

1. Control에서 catalase 活性은 呼吸作用時보다 光合成作用時에 一般的으로 減少되었다. 이러한 傾向은 가스處理時에 더욱 顯著하였다.

2. 同一濃度의 가스處理時에 單時間處理보다 長時間處理로 因한 catalase 活性의 減少는 더욱 顯著하였다.

3. 가스로 因한 植物體의 被害는 同一條件에서 呼吸作用에서 보다 光合成作用時에 더욱 높았다.

4. SO_2는 catalase 活性의 inhibitor임을 알 수 있었다.

References

Agner, K. and H. Theorell. 1946. *Arch. Biochem.*, 10; 321.

Amberger, A. and A. Wünsch. 1963. Phlanzenphysiologische wirkungen des cyanamide, *Landwirtschaftliche Forschung*. 16(12). 162—172.

Chance, B. 1951. The Enzyme, in J.B. Rummer and K. Myrbäck. New York Academic Press. 2(1). 428—453.

Euler, H.V. and K.Josephson. 1923. *Ber.*, 56. 1749.

Fumia, Y. and M. Hirokazu. 1972. Distribution and reaction of sulfur dioxide after absorption by plants. *J. Sci. Soil & Manure*, Japan. 43(7). 245—250.

Keilin, D. and E.F. Hartree. 1945. *Biochem. J. London.* 39. 293—301.

Martin Frobischer, 1968. Fundamentals of Microbiology. W.B. Saunders Co. 375—402.

Sung, M.W. 1973. Effects of ammonia on the sulfur dioxide injuries in plants. *Kor.J.Bot.* 16, 17—22.

Taniyama. T., H. Arikado, Y. Iwata and K. Sawanaka. 1972. Studies on the mechanism of injuries effects of toxic gases on crop plants. *Pro. Crop Sci. Soc. Jap.* 41(2). 121—125.

Thomas, M.D. and G.R. Hill. 1937. Relation of sulfur dioxide in the atmosphere to photosynthesis and respiration of alfalfa. *Plant Physiol.* 12. 309—383.

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